one-compartment macroscopic electrodiffusion model including both ion diffusion and an effective fixed charge in the pore can properly describe the selectivity of the channel. Using Brownian Dynamics (BD) simulations performed on plant and mammalian VDACs we also proposed a comprehensive detailed model of the molecular mechanism of ion permeation.

1560-Pos Board B511

Intracellular C1 as a Signaling Molecule that Potently Regulates Na and HCO_3 Transporters

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Cl^- and HCO_3^- are the two major cellular anions. Surprisingly, a role of intracellular Cl^- (Cl^-_{in}) as a signaling molecule that regulates the activity of other transporters has not been evaluated. We found that Cl^-_{in} functions as a regulator of cellular Na^+ and HCO_3^- concentrations by regulating the activity of several NBCs. All forms of regulation by Cl^-_{in} are mediated by the Cl^- interacting GXXXP motifs. In the basal state NBCe1-B is inhibited by high Cl^-_{in}, while NBCe1-A is resistant to Cl^-_{in} between 5-140 mM. By contracts, the IRBIT-activated NBCe1-B and the basal activity of NBCe2-C are inhibited by high affinity Cl^-_{in} sites, with apparent affinity of about 10 mM. The NBCe1-B high and low affinity Cl^-_{in} sites are mediated by separate GXXXP motifs. Mutations in the GXXXP motifs in the autoinhibitory site of NBCe1-B and the GXXXP motif in the N terminus of NBCe2-C eliminated inhibition by low Cl^-_{in}, while sparing inhibition of NBCe1-B by high Cl^-_{in}. Mutation of a second N terminus GXXXP motif was required to eliminate inhibition of NBCe1-B by Cl^-_{in}. Deletion of residues 29-41 of NBCe1-A uncovered inhibition by Cl^-_{in} that was mediated by a hidden GXXXP motif homologous with the NBCe1-B low affinity site. These finding reveal a novel Cl^-_{in} sensing mechanism that is transmitted by regulation of Na^+ and HCO_3^- transporters with major implication for cellular Na^+ and HCO_3^- homeostasis and epithelial fluid and electrolyte secretion.

1561-Pos Board B512

Sirna Knockdown of ClC-7 Produces a Lysosomal pH Increase

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Lysosomes are the terminal organelle in the endocytic pathway and have a characteristic internal pH that must be maintained for proper function. The V-type ATPase, present in all organelles in the endocytic pathway, is responsible for pumping protons into the lumen, which generates a voltage that must be dissipated by the ''counterion pathway.'' The counterion may be a cation moving out, an anion moving in, or a combination of the two. In lysosomes, Cl^- is a prominent candidate counterion because ClC-7, a chloride/proton antiporter, is located in the lysosomal membrane. However, ClC-7's contribution to acidification remains controversial. Previous qualitative siRNA knockdown experiments in HeLa cells indicate a pH increase in lysosomes with ClC-7 knocked down, but experiments with knockout mice have indicated no pH change when comparing wild type and ClC-7 knockout lysosomes. To further study this, we developed a robust and quantitative method for pH measurement in live cells using the fluorescent dye Oregon Green 488 covalently attached to dextran. Dextran is shuttled along the endocytic pathway and accumulates in lysosomes, but does not get degraded by lysosomal enzymes. Oregon Green 488 has two excitation wavelengths that are used: excitation at 490nm is strongly pH sensitive, while excitation at 440nm is pH stable, providing a ratiometric reference standard. Using a pool of four siRNA molecules to knock down ClC-7 in U-2 OS cells, we observed two populations of lysosomes: one with a pH similar to untransfected cells and one with a significantly higher pH. Control siRNA experiments showed no increase in lysosomal pH, indicating that the increase observed is due to the ClC-7 targeting siRNA. Knocking down Ostm1, the β-subunit of ClC-7, produced similar results to those of the ClC-7 knockdown, confirming that ClC-7 is necessary for proper pH maintenance.

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An Un-Bleachable YFP-Based Chloride Sensor

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Prestin (SLC26A5), the protein responsible for electromotility in the mammalian outer hair cell, remains incompletely characterized. We are interested in the protein's transporter/channel-like behavior. Clearly, the functional analysis of many chloride-related membrane proteins, such as channels/transporters/receptors, would benefit from a genetically encoded chloride sensor with reliable properties. Recently we developed an YFP-based chloride sensor, mCl-YFP (Sheng et al., PLoS ONE 2014, 9(6): e99095), with chloride Kd of 14 mM and pKa of 5.9, as well as 15-fold better photostability than wild-type EYFP. Using this chloride sensor, we demonstrated enhanced dynamic flux of chloride in the mM range into HEK cells expressing the fused protein of prestin and mCl-YFP. In order to avoid photobleaching interference during long exposure measurements, a new chloride sensor was developed with 80-fold better photostability than wild-type EYFP by stabilizing the chromophore structure via hydrogen-bond network. Other characteristics are similar to the original sensor we developed. We are currently testing the new sensor's sensitivity to other anions that typically exist in cells. We expect that this sensor will provide more detailed information on the transporter function of prestin, as well as other membrane proteins.

Systems Biology and Disease

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How Bad is it Doc? the Varying Predictions of ODE Cancer Growth Models

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Cancer is the second most common cause of death in the US. While mathematical models are often used to predict progression of the disease and treatment outcomes, we don't yet know how to accurately model tumor growth. Several ODE models (exponential, Mendelsohn, logistic, linear, surface, Bertalanff y, and Gompertz) have been proposed to model tumor growth. We examined the predictions of the models both for untreated growth and growth during treatment with chemotherapy. We compared the predicted maximum volume of the tumor, the doubling time, and the amount of chemotherapy needed to end tumor growth for each of the models and found that the predictions vary dramatically. This work highlights the need for detailed experiments to aid in choosing appropriate cancer growth models.

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Spatial Organization of Ribosome Biogenesis of Escherichia Coli

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In living cells, ribosomes are the factories that translate proteins from mRNA. Ribosomal RNAs are synthesized through the transcription of seven ribosomal RNA operons(*rrnA, rrnB, rrnC, rrnD, rrnE, rrnG, rrnH)*, which contain 99% identical genes encoding the 16S, 23S, and 5S rRNAs, and one or several tRNAs. Despite thorough investigation of the structures and functions of ribosomes, the biogenesis of ribosomes in vivo remains poorly characterized. Since *E. coli* can grow with only one intact rRNA operon with surprisingly little growth defect, the reasons for the presence of seven closely identical copies of the rRNA operons and their possibly distinguishable contributions to the total complement of ribosomes are not yet answered. In this work, we will develop a system to label and distinguish the individual operons while simultaneously determining the amount and spatial distribution of the ribosomes generated by each operon from the other ribosomes. The rRNA operon loci are labeled by the fluorescent repressor-operator system. In order to distinguish between ribosomes, a short sequence is inserted into a phylogenetically variable region of the 23S rRNA of the target rRNA. Single molecule FISH (smFISH) probes targeted at this inserted sequence enable the super resolution imaging of the ribosomes generated from the modified rRNA. The system allows measuring the ribosome output of each operon in different growth state and its kinetics of switching between growth states and therefore will further reveal the biogenesis of ribosomes and its possible contributions to determining global patterns of gene expression.

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Spatial Symmetry Breaking Determines Spiral Wave Rotation Direction in Simplified Cardiac Systems

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Chirality (''rotation direction'') represents a fundamental property of spiral waves, self-sustaining vortices of electrical activity thought to underlie a number of re-entrant cardiac arrhythmias. However, the key regulators of spiral wave chirality remain unclear. We introduced inexcitable obstacles into cardiac monolayers composed of embryonic cardiac chick cells, which gave rise to clockwise-rotating, counterclockwise-rotating, and pairs of spiral waves. Numerical simulations of the experiments showed that the precise location of the obstacle in combination with the pacing frequency determined spiral wave chirality [1]. In particular, we observed that obstacle position controls